DEVELOPMENT OF EMBRYO OF GNETUM

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(WITH PLATES XXXIX–XLI AND ONE FIGURE)

In his paper on the morphology of *Gnetum*, THOMPSON (6) fully described the different phases in the reproduction of this genus, except the development of the embryo. The material for an investigation of the latter subject was turned over to me, and the results of my study are given in the following pages.

The species studied included *G. funiculare*, and *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 of the Buitenzorg Botanic Garden. Certain stages were also studied in *G. Gnemon*. The young stages and thicker parts of the embryo were cut in serial section. Preparations of the ripe red fruit and germinating seeds of *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 were made by dissecting out the embryos with their tortuous suspensors, which were then stained in Delafield's haematoxylin, extended, and mounted in balsam. On account of the widely branching character of the suspensors this method could not be used for *G. funiculare* and *G. Gnemon*, and it was necessary to make serial sections.

History

BOWER (1) gives the following account of the history of the work on the embryo of *Gnetum*, prior to his own study of the subject. In 1832 BLUME and GRIFFITH had observed in *G. scandens* and *G. latifolium* respectively that within a cavity in the endosperm a coiled mass of suspensors is formed bearing an embryo with two small cotyledons. HOOKER examined sections of *G. Gnemon* and found therein "tubular cells" which occasionally branch, permeating the apical part of the endosperm.

In 1882 BOWER published his account of the development of the embryo of *G. Gnemon*. He states that in the ripe seeds the suspensors bear no embryo, but have the appearance described by HOOKER. Although doubtful of the origin of the first cell of the embryo, formed after germination, he assumes that it is cut

off from the apex of the suspensor. Then he describes the formation of the multicellular embryo, in which an apical cell functions. No mention is made of long secondary suspensors, such as those observed in *G.* sp. 15 and *G.* sp. 59; but the description of the differentiation of the tissues in the embryonic body is in accord with that given later for these species. Reference is also made to seeds of an unknown species in which the suspensors form a coiled bundle in a cavity in the endosperm. This corresponds to the condition found in the ripe fruit of *G.* sp. 29; but it is stated that in the individual suspensors a single large nucleus appears toward the tip of the tube, and no reference is made to the peculiar cell which is present in *G.* sp. 29.

LOTSY examined *G. Gnemon* in 1899. He described the extensive suspensor-like elongation of the fertilized egg, the branching of this tube, and the cutting off of an embryo cell at the tip.

COULTER (**3**) reinvestigated the early stages in the development of the same species in 1908. He found that in the formation of the suspensor free nuclear division takes place, resulting in a few nuclei scattered along the suspensor, which are often separated by transverse walls. He describes a terminal embryo cell containing one of the free nuclei which continues to divide, accompanied by cleavage walls until a multicellular embryo is formed. This account differs from that given by BOWER in this respect, that the earlier writer described an apical cell as functioning, and no free nuclear division.

In 1916 THOMPSON (**6**) published observations on the embryo of several species of *Gnetum*. Differing from LOTSY'S account, he states that the fertilized egg of *G. Gnemon* divides into two cells, both of which develop into suspensors without transverse cleavage walls. In *G.* sp. 33 he describes a proembryo consisting of a small group of irregularly arranged cells, produced by division of the fertilized egg. Each of these cells then elongates, forming a tortuous suspensor which contains normally only one nucleus, although the possibility of the occurrence of several nuclei would not be excluded in all cases. Finally, he observed the suspensors of *G. moluccense* growing outside of the endosperm, between it and the nucellus. He states that the tips of the tubes enlarge within the

endosperm, and a group of cells is figured at the end of one of the suspensors,

Description

The early post-fertilization stages have not been satisfactorily determined by the writer, who has not observed the number of divisions of the fusion nucleus. The earliest stage which was recognized is that represented in the reconstruction of *G. funiculare* (fig. 1). This shows a large single-celled proembryo, from several points of which there have grown suspensors. Fig. 3 shows a later stage. While some of these tubes grow directly through the endosperm, others turn over the upper part of the embryo sac and make their way down the opposite side. These suspensors often form a tangle in the narrow part of the embryo sac. THOMPSON has stated that in *G. moluccense* suspensors are found between the endosperm and the nucellus. Several times the writer has observed the same condition in *G. funiculare*. In this species sometimes a tangle of tubes is observed in that position. At certain distances, cross-walls are formed in these suspensors, and branches grow into the endosperm (fig. 17). The ends of the suspensors in the endosperm have the usual dense protoplasm and nucleus.

In the earlier stages of the development of the embryos of *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the endosperm becomes rather disorganized by the growth of tubes, so that in the ripe seed a central corrosion cavity is found. Packed in this is a coiled bundle of tubular structures (fig. 4). When these tubes are extended they measure about 23 mm. They have thick walls of a gelatinous nature. In *G. Gnemon* and *G. funiculare* no such coiled bundle of suspensors is found; they soon separate from each other and branch widely through the endosperm. Toward the tip of the suspensor in *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the protoplasm becomes denser. It surrounds a peculiar, elongated, pear-shaped cell with a deeply staining nucleus and granular protoplasm. The apex of the cell is closely applied to the wall of the suspensor (fig. 9). A short distance from this end cell there appears an extremely large nucleus with a dense nucleolus showing one or more dark colored globules (fig. 9*a*). Sometimes two similar but smaller nuclei, closely associated, may be observed before the end cell is differ-

entiated, one of which may function in the formation of that cell. There are no nuclei distributed along the suspensors.

The condition of the tubes just described is that found in the ripe fruit on the trees, and in many of the seeds on the ground. At the next stage the suspensors swell to about five times their original size, and sometimes curve about as in fig. 5. The end cell widens, and moving out from the surrounding protoplasm crowds into a protrusion of the gelatinous wall of the tube (fig. 10). Division takes place first into two cells (fig. 11); then each of these divides again, forming the four cells shown in fig. 12. In this preparation one of the walls is not visible. The cells continue to divide in an irregular manner, forming an ovoid group (fig. 13). Division continues with elongation of the basal cells. The result is the formation of a long ribbon of tissue (fig. 14) which, like the primary suspensors, is folded in the cavity of the endosperm. This secondary suspensor, usually measuring about 13 mm., is formed of rather long thin-walled cells with large nuclei. This multicellular ribbon is very different from the secondary embryonal tubes of Abietineae, and, on the other hand, is not found at all in *G. Gnemon*, according to BOWER and COULTER. At the basal end of this secondary suspensor a chain of smaller cells with denser nuclei is differentiated, which may function in the proliferation of embryos. At the apex of the secondary suspensor the cells are actively meristematic (fig. 15). When the suspensor has lengthened sufficiently, it is the rapid multiplication of this group of cells which forms the massive embryonic body.

This embryonic body differentiates in a manner similar to that described for *G. Gnemon* by BOWER (1). Fig. 22 shows the first stage as it appears externally. At the apex a conical projection is visible, surrounded by a thick ridge, from which the cotyledons develop. Fig. 23 shows the "foot" or "feeder" projecting from the side of the hypocotyledonary stem. In fig. 24 both hypocotyl and "foot" have elongated, the latter having exceeded the former. Growth continues as shown in fig. 25, and the radicle reaches the micropyle. After forcing its way out of the endosperm, the radicle turns downward. Then the hypocotyledonary stem grows rapidly and makes its way out of the endosperm also. Following this, the

cavity is filled in around the "foot," which remains in the seed as an absorbing organ, so that the condition shown in fig. 26 results.

Sections of these embryos show that internally a root tip is differentiated in a position shown by the triangle *r* in fig. 19. A definite epidermis covers the cotyledons, hypocotyledonary stem, and conical apex of the stem. After the procambium bundles have differentiated, the foot or feeder is formed as a lateral protuberance of the hypocotyledonary stem, into which there is a lateral extension of the tissues. The procambium bundles make a deep loop toward the apex. In sections of an embryo at the stage shown in fig. 21, conspicuous rows of cells, forming the laticiferous ducts, are observed. In the region of the root tip these cells are distinguished by their dense contents (fig. 27). Toward the apex they are much longer (fig. 28), but have straight end walls in which thickened portions were not observed. These correspond to the younger ducts in *G. Gnemon* figured by BOWER. Young sclerenchyma cells, with the characteristic paired nuclei, are also present in these sections.

Polyembryony

It has been stated by BOWER that polyembryony is the rule in *Gnetum Gnemon*, as in other members of the group. From the examples of it observed in *G.* sp. 15 and *G.* sp. 59 it is plain that these species are not exceptions. Some of the variations of it are represented by the diagrams in text-fig. 1. In *A* three of the four primary suspensors have developed extensive multicellular secondary suspensors measuring about 13 mm. These three have grown so closely together that at this length one has not established itself as the successful embryo, although the middle one is slightly in the lead. In *B* are shown two competing embryos keeping pace at a length of about 16 mm. The third, having separated from the others, has become aborted at a length of 6 mm., while a secondary suspensor has not developed from the other tube. In *C* only one primary suspensor has developed a secondary one. This one has already become quite massive in structure, indicating that it is to become the mature embryo. A variation of this is shown in *D*. Here two of the embryos have developed secondary suspensors, but one is stunted, so that it is quite evident which is destined to

become the successful embryo. A case of the development of several embryos from one suspensor is represented by *E*. One of the secondary suspensors has developed into a bulky structure with unusually large cells. Toward the apex of this tissue, a number of groups of meristematic cells appear which resemble normal embryos in their development. The formation of these embryos is shown

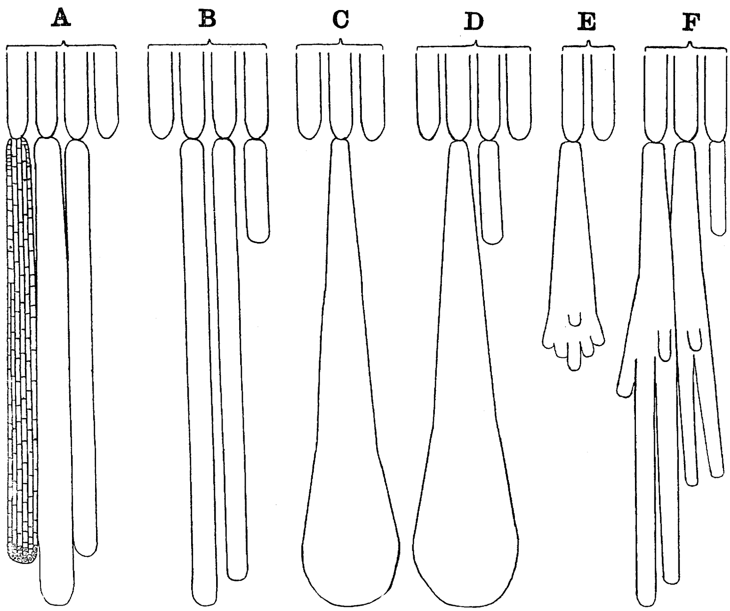

FIG. 1.—Diagrams illustrating polyembryony in *G.* sp. 15 and *G.* sp. 59: upper tubes represent primary suspensors; those below secondary suspensors; *H* and *D* represent usual type; *B* and *C* represent appearance found in small percentage of seeds; *E* and *F* show cases of proliferation of embryos, which are rarely observed.

in fig. 15, which is a small area of fig. 15*a* under greater magnification. The last diagram represents a branching of the secondary suspensor to form extra ones. In the preparation showing this condition the cells are indistinct, but the branches may be considered as prolonged "buds," such as those shown in *E*.

As already mentioned, chains of smaller cells may be observed in the tissues of the secondary suspensors. From these, in a number of cases, outgrowths have been observed which resemble a normal

embryo in development (figs. 6, 7). These cases of budding of embryos represent a process which, according to BUCHHOLZ (2), does not occur in the Abietineae. Also, although the secondary suspensors are often closely associated, no case was observed in which they fused to form a single embryo, a process described by BUCHHOLZ as normal in the higher Abietineae, but absent in the pine. From the material examined, it is concluded that in *G.* sp. 15 and *G.* sp. 59, as in other species of the genus as well as in *Ephedra*, (LAND 5), although a number of embryos may begin to develop, only one reaches maturity.

Discussion

In respect to the long coiled bundle of primary suspensors, *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 are more like the Conifers than such species as *G. Gnemon*, in which the suspensors are widely separated in the endosperm. Furthermore, it is to be noted that, while in *G. Gnemon* the embryo develops directly on the end of the primary suspensor, in the other species studied a long multicellular secondary suspensor is produced, corresponding, although different in form and size, to structures present in the Abieteneae. In all species the development of the external form, as well as of the tissues of the embryo proper, is similar and resembles that described for *Welwitschia* (4).

In certain features of the gametophyte and endosperm THOMPSON has shown that *G. Gnemon* is quite different from the other species of the genus, and nearer the angiosperms. It is of course also distinct in its arboreal habit, for the others are all vines. The present study shows that it is different from several other species in the development of its embryo. No long secondary suspensor is formed, and the primary suspensors ramify widely through the endosperm. The reduction in the suspensors appears to bring it nearer the angiosperms. The great reduction in the amount of free nuclear division is a character which separates *Gnetum* widely from the lower gymnosperms. COULTER and BOWER differ as to whether there is any free nuclear division in the formation of the embryo proper at the end of the primary suspensor.

There is certainly none in this position in the other species studied by the writer.

BUCHHOLZ concludes that cleavage polyembryony is a primitive condition in the Abietineae. It is therefore interesting to note that certain species of *Gnetum* retain this condition. There is no fusion of suspensors resulting in a reduction of the number of embryos, such as BUCHHOLZ finds in the higher Abietineae and regards as an evolutionary development. The occasional formation of supernumerary embryos by splitting is evidently of no fundamental significance.

Summary

1. The proembryo of *G. funiculare* consists of a single cell from which suspensors emerge in different directions.

2. Cross-walls and nuclei are formed in these tubes, associated with the branches.

3. In *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the suspensors form a coiled rope in a cavity in the endosperm; while in *G. funiculare*, as in *G. Gnemon*, they branch widely through the endosperm.

4. In the ripe seeds a peculiar cell is present at the end of the primary suspensor.

5. When germination begins in *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59, a very long multicellular secondary suspensor is formed by division of this cell at the tip of the tube. In *G. Gnemon* no such body appears.

6. The development of cotyledons, root tip, and "foot" at the end of the secondary suspensor is described.

7. Polyembryony is the rule. Several of the primary suspensors usually form secondary suspensors. In some cases these are closely associated and develop equally for some time. In other cases one of the suspensors wins out at an early stage, and the others separate and become stunted. Occasionally the tip of the secondary suspensor divides into a number of branches. Branches from the side of a secondary suspensor are sometimes observed.

8. Except in the reduction of the amount of free nuclear division in all species and in the suspensors in *G. Gnemon* the development is gymnospermic.

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EXPLANATION OF PLATES XXXIX–XLI

PLATE XXXIX

Fig. 1.—*G. funiculare:* proembryo, showing suspensors emerging; *S*, suspensors; *pt*, pollen tube; semi-diagrammatic reconstruction; ×50.

Fig. 2.—*G. funiculare:* proembryo, showing variation of fig. 1; semi-diagrammatic reconstruction; ×50.

Fig. 3.—*G. funiculare:* branching of tubes; *oe*, outside of endosperm; connections in broken lines not determined with certainty; semi-diagrammatic reconstruction.

Fig. 4.—*G.* sp. 29: part of bundle of tubes measuring 23 mm.; ×25.

Fig. 5.—*G.* sp. 15: swollen tips of tubes turning about; ×25.

Fig. 5*a*.—*G.* sp. 15: group of nuclei from tube marked *X*; ×475.

Fig. 6.—*G.* sp. 59: embryo forming from chain of cells in secondary cells (*S*) of secondary suspensor; ×215.

Fig. 7.—*G.* sp. 59: branch of secondary suspensor; ×107.5.

PLATE XL

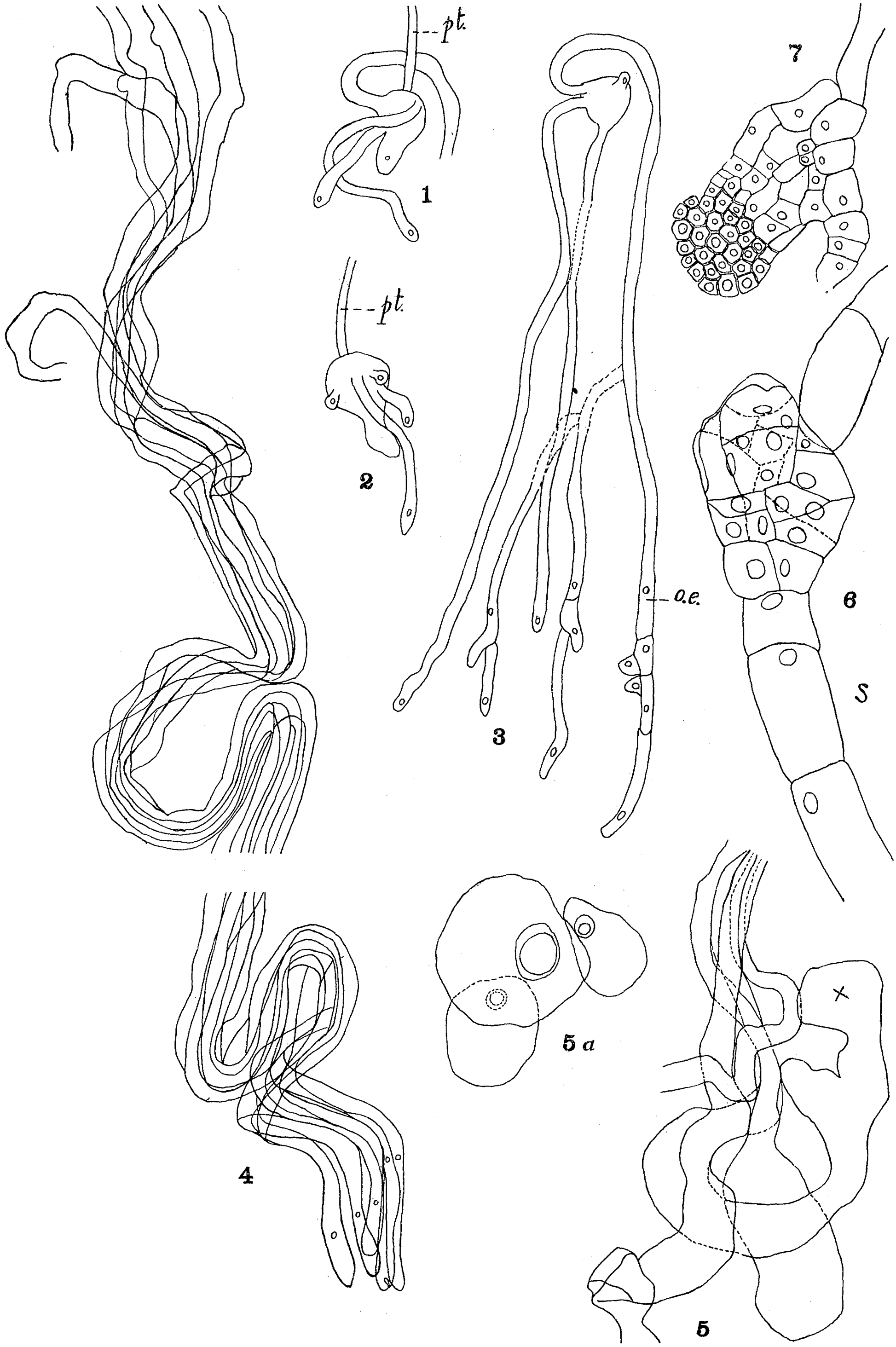
Fig. 8.—*G. funiculare:* branch of primary suspensor in endosperm; ×50.

Fig. 9.—*G.* sp. 29: tip of primary suspensor, showing peculiar cell; ×425.

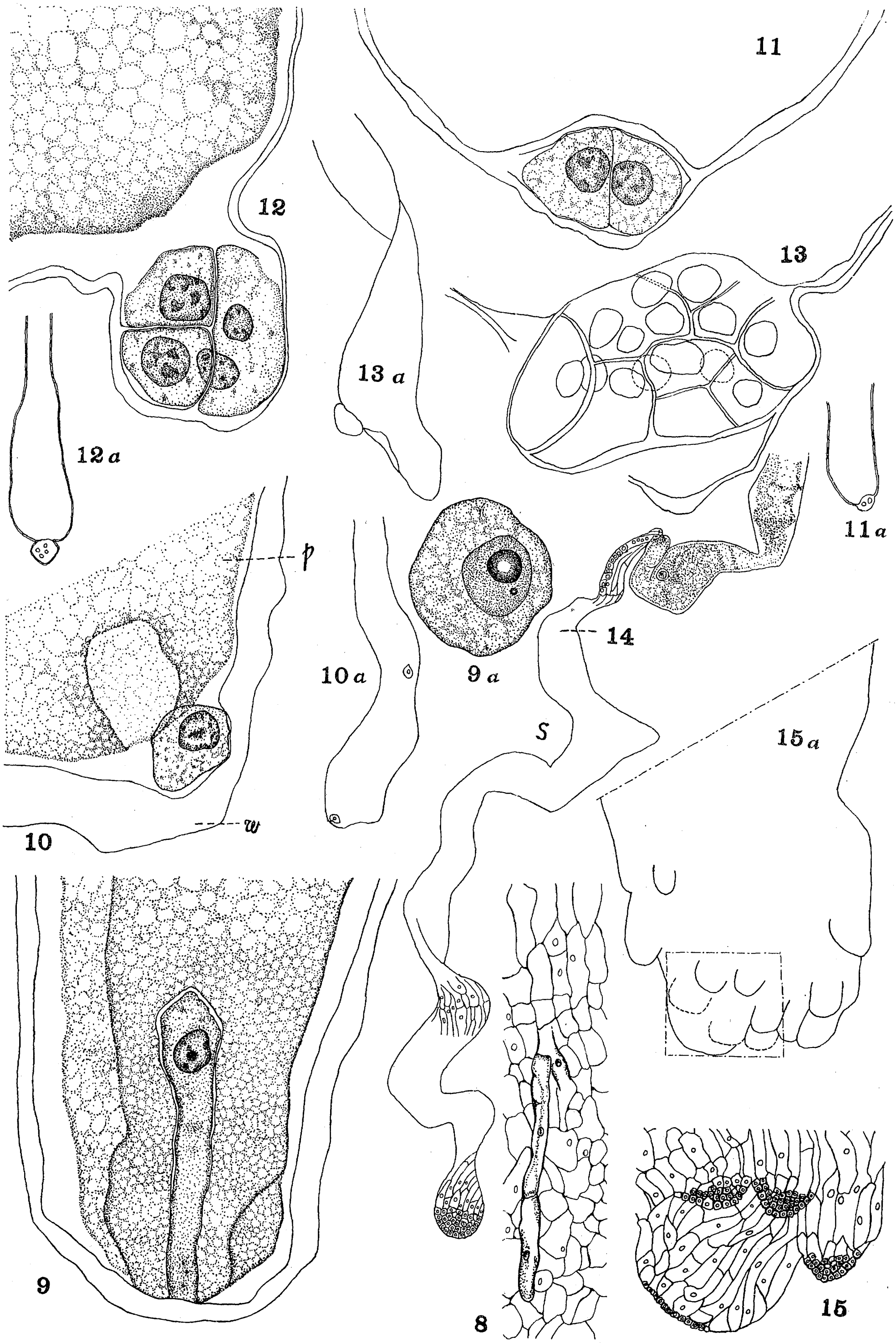
Fig. 9*a*.—*G.* sp. 29: large nucleus of suspensor shown in fig. 9; ×425.

Fig. 10.—*G.* sp. 59: shortened end cell moving out of surrounding protoplasm; *p*, shrunken protoplasm of tube; *w*, wall; ×425.

Fig. 10*a*.—*G.* sp. 59: same under lower magnification; ×50.



HAINING on GNETUM



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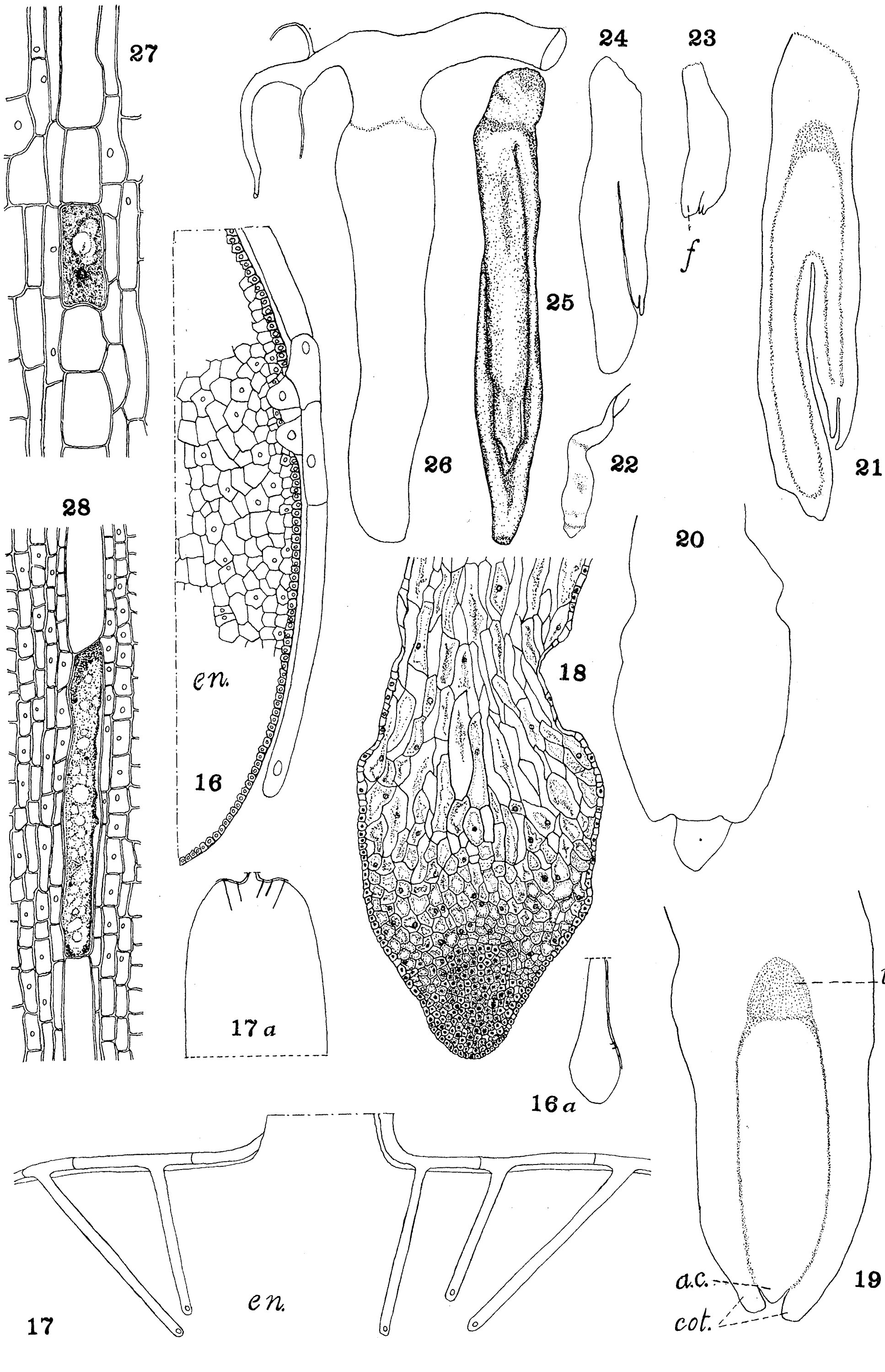

HAINING on GNETUM

FIG. 11.—*G.* sp. 15: 2-cell stage of secondary suspensor; tube placed to underlie these cells; ×425.

FIG. 11*a*.—*G.* sp. 15: same tube under lower magnification; ×50.

FIG. 12.—*G.* sp. 15: 4-cell stage; ×425.

FIG. 12*a*.—*G.* sp. 15: same tube under lower magnification; ×50.

FIG. 13.—*G.* sp. 59: group of cells irregularly formed; walls too faintly stained to see underlying ones; ×425.

FIG. 14.—*G.* sp. 15: swollen end of primary suspensor, showing ribbon of secondary suspensor; *s*, secondary suspensor; ×25.

FIG. 15.—*G.* sp. 59: groups of meristematic cells at the apex of secondary suspensor tissue; ×50.

FIG. 15*a*.—*G.* sp. 59: same under lower magnification, showing location of groups; ×25.

PLATE XLI

FIG. 16.—*G. funiculare:* suspensor outside endosperm, in immature seed, budding branches; semi-diagrammatic reconstruction; *en*, endosperm.

FIG. 16*a*.—*G. funiculare:* embryo sac, showing position of tube in fig. 16; semi-diagrammatic; ×5.

FIG. 17.—*G. funiculare:* branches penetrating endosperm from tangle of tubes without; in ripe fruit; diagram *en*, endosperm.

FIGS. 18–28.—*G.* sp. 15.

FIG. 18.—Section of tip of secondary suspensor, showing group of meristematic cells and structure of tissue; ×2.5.

FIG. 19.—Section of body of embryo showing stage before "foot" is differentiated; *cot*, cotyledon; *ac*, central apical cone; *r*, root tip; ×25.

FIG. 20.—Section of body of embryo; ×25.

FIG. 21.—Section of older embryo; ×7.5.

FIG. 22.—Embryonic body: gross material; ×7.

FIG. 23.—Embryonic body: cotyledons and "foot" (*f*) differentiated; gross material; ×7.

FIG. 24.—Same, later stage; ×7.

FIG. 25.—Same, later stage; ×7.

FIG. 26.—Root and hypocotyledonary stem outside of seed; ×7.

FIG. 27.—Longitudinal section of young laticiferous cells in basal part of embryo; ×107.5.

FIG. 28.—Longitudinal section of laticiferous cells from nearer apex; ×107.5.